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Your reference MTW-50632/GB

Patent application number *(The Patent Office will fill in this part)* 07 JUL 1998 **9814653.3**

Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 4329587002

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Title of the invention

METHOD OF REDUCING OR PREVENTING MALODOUR

Name of your agent *(if you have one)*

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MTW50632

METHOD OF REDUCING OR PREVENTING MALODOUR

This invention relates to perfume components, mixtures thereof and perfume compositions, to personal products and detergent products containing such perfume components, and to the use of such perfume components to deliver a deodorant effect.

In particular, it relates to perfume components, mixtures thereof, and perfume compositions for inhibiting the production of odorous metabolites by topically applying to human skin perfumery components capable of inhibiting the production of body malodour caused by micro-organisms comprising corynebacteria, preferably by selectively inhibiting those corynebacteria capable of catabolising fatty acids.

It is well known that freshly secreted sweat is odourless and that body malodour is the result of a biotransformation of the sweat by micro-organisms living on the surface of the skin to produce volatile odoriferous compounds.

There are three types of personal product routinely used to combat body malodour: perfumes, antiperspirants and deodorants.

Perfumes may simply mask body malodour. However perfume compositions have been disclosed which exhibit a deodorant action. EP-B-3172, EP-A-5618, US-A-43044679, US-A-4322308, US-A-4278658, US-A-4134838, US-A-4288341 and US-A-4289641 all describe perfume compositions which exhibit a deodorant action when applied to human skin or when included in a laundry product used to launder textiles.

Antiperspirants work by blocking the sweat glands thereby reducing perspiration.

Antimicrobial agents used in deodorants are designed to reduce the population of micro-organisms living on the surface of the skin. Typical agents of this nature include ethanol and Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl ether) which are well known to exert antimicrobial effects. The use of common deodorant actives results in a non-selective antimicrobial action exerted upon most of the skin's natural microflora. This is an undesirable side effect of such deodorant formulations.

Many disclosures describe compositions comprising antimicrobials which are designed to eliminate malodour by sub-lethally reducing the microflora population.

WO 95/16429 (Henkel) describes deodorant compositions comprising fat soluble partial esters of hydroxy carboxylic acids.

WO 95/07069, WO 91/11988 and WO 91/05541 (all Gillette) describe deodorant compositions comprising inhibitors of pyridoxal phosphate dependent amino acid lyase.

WO 94/14934 (Unilever) describes a method for reducing the perceptibility of an odoriferous substance using an antibody or antibody fragment. Such antibodies could be used in deodorant compositions.

WO 93/07853 (Monell) describes the use of mimics of the odoriferous compound 3-methyl-2-hexenoic acid to reduce body malodour.

DD 29 39 58 (Medezinische Fakultaet (Charite) der Humboldt Universitaet zu Berlin) describes the use of lipoxygenase inhibitors to act biochemically to reduce sweat production

or to inhibit, to various degrees, the action of skin bacteria or their enzymes on the decomposition of sweat to form unpleasant-smelling substances.

DE 43 43 265 (Henkel) describes deodorant compositions comprising saturated dioic acid (C3 - C10) esters. It is claimed that the active inhibits a sweat decomposing esterase and the compositions are said not to disturb the skin's natural microflora.

DE 43 43 264 (Henkel) describes the use of lipid-soluble partial esters of hydroxy carboxylic acids in deodorant compositions.

Some disclosures describe the use of antimicrobial substances which are selective against odour producing bacteria.

WO 90/15077 (Gillette) describes the use of antibodies to a carrier or transport protein of coryneform and staphylococci. It is disclosed that these bacteria types have an amino acid lyase enzyme which is responsible for the formation of malodour.

DE 43 39 605 (Beiersdorf) describes the use of deodorising mixtures of alpha-omega alkanedioic acids and fatty acid partial glycerides of unbranched fatty acids which may be present in a suitable cosmetic vehicle to combat Gram-positive, particularly coryneform, bacteria.

Woolwax acids have also been disclosed in the following Beiersdorf publications as deodorant actives in combination with:

-alpha-omega alkanedioic acids (DE 43 24 219);

-partial glycerides of unbranched fatty acids (DE 43 09 372); or

-monocarboxylic acids, especially unbranched fatty acids (DE 43 05 889).

Each combination is described as suitable to combat Gram-positive, especially coryneform bacteria.

DE 42 37 081 (Beiersdorf) describes deodorant compositions comprising monocarboxylic acid diglycerides and/or triglycerides. The compositions are said to be suitable against Gram-positive, especially coryneform, bacteria.

EP-A-0 697 213 (Beiersdorf) describes the selective reduction of coryneform bacteria using a mixture of:

-lauric acid;

-one other fatty acid C6 - C20 (one of which must be at least C12);

-glyceryl monocaprate/glyceryl monocaprylate;

-without the use of ethoxylated glyceryl fatty acid esters and propoxylated glyceryl fatty acid esters;

which has a pH of less than 8.

WO 94/07837 (Unichema) describes certain novel unsaturated dioic acids having between 8 and 22 carbon atoms. Also described is their potential use to treat malodour.

EP-A-0 750 903 (Cooperatie Cosun UA) discloses deodorant compositions comprising sugar-fatty acid esters. The actives are described as being selective towards odour causing micro-organisms. These odour-causing micro-organisms are said to be the Corynebacterium varieties known as lipophilic diphtheroids such as *Corynebacterium xerosis*

and *C. minutissimum*.

Coryneform is a designation of a large ill-defined group of bacteria. The diverse genera that have been included with the coryneforms include Actinomyces, Arachnia, Arcanobacterium, Arthrobacter, bacterionema, Bifidobacterium, Brevibacterium, Cellulomonas, Corynebacterium, Eyrsipelothrix, Eubacterium, Kurthia, Listeria, Mycobacterium, Nocardia, Oerskovia, Propionibacterium, Rhodococcus and Rothia.

It is clear that the majority of previous disclosures in this area have been aimed at antibacterial or bacteriostatic effects towards the whole skin flora or selected species.

Without being bound by theory we believe that the *Corynebacterium* genus can be subdivided into two subgroups according to ability to catabolise fatty acids. We further believe that one of these subgroups, hereinafter referred to as "Corynebacteria A", which is capable of catabolising fatty acids, contributes strongly to the formation of body malodour, in particular axillary malodour. The other subgroup, hereinafter referred to as "Corynebacteria B", which catabolises fatty acids much less so or not at all, contributes much less or even not at all to malodour formation. We also believe that it is possible to selectively inhibit the generation of odorous metabolites by Corynebacteria A.

The deodorants available on the market tend to be insufficiently effective and/or substantially reduce the numbers of all bacteria in the microflora indiscriminately. The present invention offers the opportunity to provide deodorant products which for many females will substantially reduce malodour formation while inhibiting only a minor portion of the microflora. For many males malodour formation can be substantially reduced or even largely eliminated by inactivating the Corynebacteria A.

Furthermore, we have found a range of perfume components capable of selectively inactivating Corynebacteria A, while leaving other bacteria, notably Corynebacteria B much less affected or even not notably affected at all. Significant deodorant action can be obtained by the action of these components singly or in combination.

Accordingly, the invention provides a cosmetic method for reducing or preventing body malodour by topically applying to human skin at least one perfume component capable of inactivating malodour-causing micro-organisms comprising corynebacteria. Compositions useful in the method of the invention contain at least one perfume component which is capable of inactivating the Corynebacteria which are capable of catabolising fatty acids.

The invention also comprises a perfume composition comprising a perfume component selectively capable of inhibiting the metabolic pathway of corynebacteria, wherein the perfume component is capable of inactivating the corynebacteria capable of catabolising fatty acids and the use of such compositions to reduce body malodour.

The term "perfume component" is used herein to represent a material which is added to a perfume to contribute to the olfactive properties of the perfume. A perfume component can be acceptably employed to provide odour contributions to the overall hedonic performance of products. Typically, a perfume component will be generally recognised as possessing odours in its own right, will be relatively volatile and often has a molecular weight

within the range 100 to 300. Typical materials which are perfume components are described in "Perfume and Flavour Chemicals", Volumes I and II (Steffan Arctander, 1969). A perfume composition will contain a number of individual perfume components, and optionally a suitable diluent.

The perfume components used in the current invention inactivate Corynebacteria, preferably selectively inactivating Corynebacteria A. Inactivation may mean killing the bacteria, i.e. a bactericidal effect but preferably the effect is sub-lethal, i.e. bacteriostatic, preferably meaning that a viability of at least 80%, preferably 90% and, especially, 95% is maintained, for example, in the test method according to Example 2 herein. In particular, by sub-lethal is meant a significant inhibition of metabolism, e.g. pentadecanoic acid utilisation (at least 60% inhibition), without concomitant reductions in cell viability (not more than 1 $\log_{10}$ CFU/ml reduction) and glucose utilisation (not more than 10% reduction).

The perfume components used in the method of the invention are frequently incorporated in to deodorant products which include, but are not limited to, body deodorants and antiperspirants including roll ons, gel products, stick deodorants, antiperspirants, shampoos, soap shower gels, talcum powder, hand cream, skin conditioners, sunscreen, sun tan lotion, skin and hair conditioners.

The perfume components may also be usefully employed for deodorant properties by incorporation in to other products, for example, in laundry and household products such as rinse conditioners, household cleaners and detergent cleaners. The perfume components can be incorporated into textiles themselves during their production using techniques known in the art, to provide deodorant protection.

It is postulated that selective inhibition of Corynebacteria A is achieved by inhibiting the metabolic pathways of the Corynebacteria A which leads to a reduction in the production of malodorous metabolites. As postulated previously the selective inhibition of the metabolic pathway of Corynebacteria A is more important than the selective inhibition of the metabolic pathway of Corynebacteria B as only the Corynebacteria A are capable of producing malodorous products.

In a preferred method according to the invention, perfume components which selectively inhibit the metabolic pathway of only those corynebacteria capable of catabolising fatty acids are used, by which is meant inactivating Corynebacteria A to a significantly higher degree than Corynebacteria B. Preferably, it means inactivating Corynebacteria A to a significantly higher degree than the majority, preferably at least 75%, more preferably at least 90% of bacteria other than Corynebacteria A constituting the skin microflora.

The levels of perfume materials used in a skin product may lead to general bacteriostatic and bactericidal effects. A skilled person responsible for formulating a finished product will be able to adjust the level to produce the desired effect in the final product.

The perfume components usually employed in the present invention are more active with Corynebacteria A than with other bacteria constituting the axillary microflora, including Corynebacteria B, when considering the selective inhibition of the metabolic pathway of the

bacteria, particularly in respect of fatty acid metabolism.

The following perfume components have been shown to selectively inhibit the metabolic pathway of Corynebacteria A, leading to a reduction of malodorous compounds. These can be used to produce a deodorant effect in consumer products.

Clearly these may be mixed with other perfume components to deliver perfumes or perfume compositions with the desired deodorant and hedonistic properties. To deliver high deodorant effects these active components preferably comprise 30% or more of the total perfume formulation by weight, more preferably at least 40% and particularly at least 60%.

(Z)-3,4,5,6,6-pentamethylhept-3-en-2-one
Mixture of diethyl- and dimethyl-cyclohex-2-en-1-one (Azarbre)
Citronellol
2-methyl-3-(4-(1-methylethyl)phenyl)propanal
Dihydroeugenol
Diphenylmethane
Tetrahydrolinalol
4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde (Empetaal)
3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde
3-(1,3-benzodioxol-5-yl)-2-methylpropanal (Helional)
α- and β-Ionone and mixtures thereof
tricyclo[5.2.1.0 2,6]dec-4-en-8-yl ethanoate (Jasmacylene)
4-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde
3-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde
Methyl iso-eugenol
2-(1,1-dimethylethyl)cyclohexyl ethanoate (Ortholate)
4-(1,1-dimethylethyl)cyclohexyl ethanoate
4 -Methyl-2-(2-methylprop-1-enyl)tetrahydropyran

The invention is illustrated by the following non-limiting examples.

EXAMPLE 1

A demonstration of fatty acid catabolism in a particular bacterial strain was determined *in vitro* using the method given below:

The *in vitro* model system, reproducing fatty acid catabolism by axillary bacteria, consisted of 250 ml baffled shake flasks, to which were added 30 ml semi-synthetic medium (see below) supplemented with fatty acid substrate (2.0 mg/ml pentadecanoic acid) This system was employed to evaluate selected potential deodorant actives (see below). Flasks were inoculated with fresh bacterial biomass, pre-grown for 24 h in TSBT (see below), to give starting optical densities (A_{590}) of 1.0 - 2.0. Following inoculation, flasks were incubated aerobically at 35°C, with agitation (130 rpm), and analysed after 24 h. Culture viability/purity was determined by TVC analysis on TSAT plates (see below) following serial dilution in quarter-strength Ringers solution.

Fatty acid levels in the flasks were determined by capillary gas chromatography (GC)

analysis. Initially, 5.0 ml aliquots from each flask were rapidly transferred into universal tubes; an internal standard (1.0 mg/ml lauric acid) was added to each universal tube and the culture medium was acidified (pH ~2) by the addition of hydrochloric acid. Liquid-liquid extraction was then carried out using 2 vol (10 ml) ethyl acetate; organic and aqueous phases were resolved by centrifugation (2000 rpm, 3 min). 2.0 ml of each organic (upper) phase was then transferred to a sampling tube prior to analysis on a Perkin Elmer 8000 (Series 2) GC fitted with a 15 m x 0.32 mm (internal diameter) FFA (nitroterephthalic acid modified PEG/siloxane copolymer) fused silica capillary column (film thickness 0.25 mm) (Quadrex). This column was attached to the split splitless injector and flame ionisation detector (FID) of the GC ; injector and detector temperatures were each 300°C. Carrier gas for the column was helium (6.0 psi), while hydrogen (17 psi) and air (23 psi) were supplied the FID. The temperature programme for fatty acid analysis was 80°C (2 min); 80-250°C (20°C/min); 250°C (5 min). Sample size for injection was 0.5 -1.0 µl. Fatty acid levels in the flasks were quantified by comparison of peak areas with known levels of both internal (lauric acid) and external (pentadecanoic acid) standards.

EXAMPLE 2

Demonstration of sub-lethal inactivation of fatty acid catabolism was performed with the following in *vitro* method.

Prior to inoculation, flasks were supplemented with selected materials, at a range of concentrations below their predetermined minimum inhibitory concentration, to determine their ability to sub-lethally inhibit fatty acid catabolism by Corynebacteria A. Stock active solutions/emulsions were prepared in semi-synthetic medium (see below), emulsions were formed by ultra-homogenisation at 24,000 rpm for ~1 min. At the end of each experiment, viability and fatty acid levels in the experimental flasks were compared to those in a control flask. Sub-lethal inhibition of fatty acid catabolism was defined as significant inhibition of pentadecanoic acid utilisation, without concomitant reductions in cell viability.

Composition of Tween-supplemented Tryptone soya broth/agar (TSBT, TSAT) used for growth/maintenance of axillary bacteria (g/l) :Tryptone soya broth (30.0), Yeast extract (10.0), Tween 80 (1.0), ± Agar (20.0). Composition of semi-synthetic medium used in laboratory systems simulating fatty acid catabolism by axillary bacteria (g/l): KH_2PO_4 (1.6), $(NH_4)_2HPO_4$ (5.0), Na_2SO_4 (0.38), Yeast Nitrogen Base (Difco) (3.35), Yeast Extract (0.5), Tween 80 (0.2), Triton X-100 (0.2), $MgCl_2.6H_20$ (0.5), Pentadecanoic acid (2.0).

The results below show the compounds that are active and inactive with regard to the inhibition of fatty acid metabolism in Corynebacteria A.

Inhibition of l ng chain fatty acid metabolism bserved	No inhibition of long chain fatty acid metabolism observed
(Z)-3,4,5,6,6-pentamethylhept-3-en-2-one	Aldehyde C11
Mixture of diethyl- and dimethyl-cyclohex-2-ene-1-one	Anisic Aldehyde
Cyclamen aldehyde	Caryophyllene
Dihydroeugenol	Cinnamic alcohol
Diphenylmethane	2H-2-chromenone
4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde	
3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde	Florocyclene 3a,4,5,6,7,7a-hexahydro-4,7-methano-1H-inden-6-yl propanoate
3-(1,3-benzodioxol-5-yl)-2-methylpropanal	4,6,6,7,8,8-hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[gamma]isochromene
Mixture of alpha and beta ionone	Hexyl cinnamic aldehyde
4-(4-hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde	
3-(4-hydroxy-4-methylpentyl)cyclohex-3-ene carbaldehyde	hexyl 2-hydroxy-1-benzene carboxylate
Methyl-iso-eugenol	Iso-e-super
Ortholate 2-(1,1-dimethylethyl)cyclohexyl ethanoate	Lilial
4-Methyl-2-(2-methylprop-1-enyl)-tetrahydropyran	Thyme red

EXAMPLE 3

The following are typical formulations which comprise an agent capable of inhibiting the production of body malodour by micro-organisms comprising Corynebacteria, and in which the agent is capable of selectively inhibiting those Corynebacteria capable of catabolising fatty acids.

These formulations are made by methods common in the art.

Deodorant Sticks

Ingredient	Content (% by weight)	
	Formulation 1A	Formulation 1B
Ethanol		8
Sodium Stearate	7	6
Propylene glycol	70	12
Perfume	1.5	2
PPG-3 Myristyl ether		28
PPG-10 Cetyl ether		10
Cyclomethicone		34
Silica		
Water	21.5	

Aerosols

Ingredient	content % by weight	
	Formulation 2A	Formulation 2B
Ethanol B	up to 100	
Propylene glycol	as required	
Perfume	2.5	1.5
Chlorhydrol microdry		31.8
Silicone Fluid DC344		up to 100
Bentone gel IPP		13.65
Irgasan DP300	0.03	
Dimethyl ether	20	
Concentrate		22
Water	23	

Roll ons

Ingredient	Content % by weight	
	Formulation 3A	Formulation 3B
Ethanol	to 100%	60
Klucel MF		0.65
Cremphor RM410		0.5
Perfume	0.5	1
AZTC*	20	
Cyclomethicone	68	
Dimethicone	5	
Silica	2.5	
Water		37.85

* Aluminium zirconium tetrachlorohydro glycinate

Three perfume compositions embodying this invention were made and tested for deodorant action in an underarm product, using an Odour Reduction Value test generally as described in US 4 278 658, but with the substitution of the perfumed soap by perfumed roll-on product, using the formulation described in Formulation 3B. These perfume compositions and the method for an odour Reduction Value Test are set out below.

	Composition by %	
	Perfume A	Perfume C
Acetyl di iso amylene	10	7
Adoxal		0.5
Amberlyn super PM 577 10%DPG	3	
Azarbre	3.5	
Benzyl acetate extra	8	8
Benzyl salicylate	8	12
Cassis base		5
Citral lemarome		3
Citronellol pure		15
Cyclamen aldehyde		5
Dihydro jasmone	0.5	
Diphenyl methane	3	
Dupical		0.3
Helional		4
Ionone	15	
Jasmacyclene	3	
Ligustral 10%DPG AAA 1486	3	
Lyral	8	15
Methyl iso eugenol	5	
Methyl octyl acetaldehyde 10%DPG AA1918		2
Ortholate		8
Para tert butyl cyclo hexyl acetate	12	
Phenyl ethyl alcohol	12	13
Roseacetone	6	2.2

	Composition by %
	Perfume E
Aldehyde C11 (undecyclic) 10%DPG AA1655	4
Anisic aldehyde	2.5
Bangalol	4
Benzyl alcohol	7.5
Cedarwood texan pure	4
Citronellol pure	12
Dipropylene glycol	14.6
Hexyl cinnamic aldehyde	25
Hydroxycitronellal	8
Karanal	0.4
Lilial	10
Lixetone	8

The Odour Reduction Value test was carried out using a panel of 40 Caucasian male subjects. A standard quantity (approximately 0.4g) of a roll-on product containing one of the perfume compositions or an unperfumed control was applied to the axillae of the panel members in accordance with a statistical design.

After a period of five hours the axillary odour was judged by three trained female assessors who scored the odour intensity on the 0 to 5 scale, as shown below.

Score	Odour level	Conc. of aqueous isovaleric acid (ml/l)
0	No odour	0
1	Slight	0.013
2	Definite	0.053
3	Moderate	0.22
4	Strong	0.87
5	Very Strong	3.57

Average scores for each test product and the control product were then determined and the score for each test product was subtracted from the score for the control product to give the Odour Reduction value.

Average panel score perfume A	2.08
Control panel score	2.31
Odour Reduction Value perfume A	0.23
Odour Reduction Value as percentage of control score	10%

Difference for significance @95% 0.21
Difference for significance @99% 0.28

Average panel score perfume C	1.98
Control panel score	2.31
Odour Reduction Value perfume C	0.33
Odour Reduction Value as percentage of control score	14%

Difference for significance @ 95% 0.21
Difference for significance @ 99% 0.28

Average panel score perfume E	2.25
Control panel score	2.31
Odour Reduction Value perfume E	0.06
Odour Reduction Value as percentage of control score	3%

Difference for significance @ 95% 0.21
Difference for significance @ 99% 0.28

For each of the perfume compositions given in Example 3 perfumes A and C contained at least or greater than 50% of active components. The perfume referred to as E contained only 20 % or less of active components.

CLAIMS

1. A cosmetic method for reducing or preventing body malodour by topically applying to human skin a composition comprising an active agent capable of inactivating body malodour-causing micro-organisms comprising corynebacteria, characterised in that the agent is a perfume component which is capable of inactivating the corynebacteria capable of catabolising fatty acids.

2. A cosmetic method according to claim 1 wherein the perfume component comprises at least one of the following materials
(Z)-3,4,5,6,6-pentamethylhept-3-en-2-one, mixtures of diethyl- and dimethyl-cyclohex-2-en-1-one, citronellol, 2-methyl-3-(4-(1-methylethyl)phenyl)propanal, dihydro-eugenol, diphenylmethane, 4-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde, 3-(4-methyl-3-pentenyl)cyclohex-3-ene-1-carbaldehyde, 3-(1,3-benzodioxol-5-yl)-2-methylpropanal, α-ionone, β-ionone, tricyclo[5.2.1.0,2,6]dec-4-en-8-yl ethanoate, 4-(4-hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde, 3-(4-hydroxy-4-methylpentyl)-cyclohex-3-enecarbaldehyde, methyl iso-eugenol, 2-(1,1-dimethylethyl)cyclohexyl ethanoate, 4-(1,1-dimethylethyl)cyclohexyl ethanoate, 4-methyl-2-(2-methylprop-1-enyl)tetrahydropyran, tetrahydrolinalol.

3. A cosmetic method according to claim 1 wherein the composition comprises at least 30% by weight of at least one of the perfume components listed in claim 2.

4. A cosmetic method for reducing or preventing body malodour by topically applying to human skin a composition comprising a perfume component which inactivates corynebacteria capable of catabolising fatty acids.

5. A cosmetic method according to any one of the preceding claims wherein the perfume component inactivates only those corynebacteria capable of catabolising fatty acids.

6. A perfume composition comprising a perfume component selectively capable of inhibiting the metabolic pathway of corynebacteria, characterised in that the perfume component is capable of inactivating the corynebacteria capable of catabolising fatty acids.

7. The use of a perfume component to reduce body malodour characterised in that the perfume component is capable of inactivating the corynebacteria capable of catabolising fatty acids.

8. The use of a perfume composition comprising a perfume component to reduce body malodour characterised in that the composition comprises at least 30% by weight of at least one of the perfume components listed in claim 2.

9. A deodorant product comprising a perfume composition according to claim 6.

ABSTRACT

A method for reducing or preventing body malodour by topically applying to human skin perfumery materials capable of inhibiting the production of malodorous metabolites caused by micro-organisms comprising corynebacteria. The perfumery materials are capable of inactivating corynebacteria capable of catabolising fatty acids.

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